Methods: VSMC grown in serum free media for 48hours were co-cultured with monocytes (M) for 72hours, in the presence of M-CSF or IL1β. The apoptotic index was measured using an established apoptosis assay. M-CSF and IL1β were used at concentrations of 100ng/ml and 100pg/ml to 1ng/ml, respectively. Appropriate controls were set up for all experiments.
Results: IL1β at physiological concentrations (100pg/ml) did not increase VSMC apoptosis (10.5±2.4%); this was similar to control VSMC with M alone, whereas M-CSF-activated M significantly increased VSMC apoptosis (60.0±3.0%, p≤ 0.008). Supraphysiological doses of IL1β (500pg and 1ng/ml) did cause an increase in monocyte-induced killing of VSMC (59.5± 3.5% and 59.0± 3.8% respectively). However, such effect was shown to be mediated by endogenous production of M-CSF upon IL1β stimulation. Thus, VSMC co-cultured with M and IL1β (500pg and 1ng/ml) were pretreated with an anti M-CSF neutralizing antibody (1μg/ml). The VSMC apoptosis was nearly abrogated in the presence of the anti M-CSF mAb (17.0± 2.1% and 13.6±1.9%, p≤0.007 and p≤0.005 respectively), when compared to VSMC co-cultured with M-CSF activated M alone. A nonspecific isotype control (IgG2a) was used and did not block the IL1 (1ng/ml) induced VSMC apoptosis (55.0±2.0%). **Conclusions:** M-CSF could function as an important rate limiting cytokine in the process of activated monocyte induced killing of VSMC, suggesting a final common pathway for atheroma plaque destabilization.

11:30 a.m.

853-5 **Arterial Injury in ApoE -/- and C57/BL6 Wild Type Mice: Evidence for Macrophage Apoptosis as a Mechanism Regulating Tissue-Factor Expression and Plaque Growth**

Randolph Hutter, Juan J. Badimon, Bernhard V. Sauter, John T. Fallon, Valentin Fuster, *Cardiovascular Institute. Mount Sinai Medical Center, New York, New York.*

Background: Macrophages undergoing apoptosis are considered contributing to progression of atherosclerotic lesions. Tissue factor is a key cell-mediated activator of extrinsic coagulation-cascade and can induce thrombus formation.
Methods: In the present study, we examined the role of macrophage apoptosis on plaque growth by comparing neointima formation in ApoE -/- (n=10) and C57/BL 6 wild type mice (n=23) in a model of femoral arterial denudation injury.
Results: Arterial injury resulted in significantly increased neointima formation in ApoE -/- mice at 4 weeks compared to wild type mice ($4.84 \pm 1.26\ mm^2 \times 10^{-2}$ *vs.* $0.73 \pm 0.03\ mm^2 \times 10^{-2}$; $P<0.01$). Apoptotic macrophages and foam cells as detected by caspase-3 expression, characteristic morphology and positive staining for MOMA-2 were found only in lesions of ApoE -/- mice and accounted for up to 33 ± 6 % of intimal cells at 4 weeks after arterial injury compared to less than 0.5 % apoptotic cells in neointima of wild type mice ($P<0.01$). Importantly, neointima size significanly correlated with the content of apoptotic macrophages in neointima (r=0.64, $P<0.01$). In addition, apoptotic macrophages were directly associated with increased cellular tissue factor expression (r=0.97, $P<0.01$) as well as reduced alpha-actin expression (r=-0.75, $P<0.01$) in neointima of ApoE -/- mice.
Conclusions: Apoptosis of macrophages and enhanced expression of cellular tissue factor in neointima correlated with increased plaque growth and change of neointima to an unstable plaque-like phenotype. These findings point to an important role of programmed cell death occuring in macrophages in modulating arterial lesion biology and controlling thrombogenic properties of intimal lesions following arterial injury.

11:45 a.m.

853-6 **Asymmetric Dimethylarginine Stimulates the Expression of Matrix Metalloproteinase-9 in Human Monocytes via Tumor Necrosis Factor-a**

Euy-Myoung Jeong, Sungyoul Hong, Jeong-Euy Park, *Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea, Center for Clinical Research, Samsung Biomedical Research Institute, South Korea.*

Background: Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase, has recently been reported as an atherogenic molecule. ADMA induces the endothelial dysfunction and influences the monocyte adhesion to the endothelial cells. We tried to investigate whether ADMA induces the release of proinflammatory cytokines and the expression of matrix metalloproteinase-9 (MMP-9).
Methods: Human monocytic cell line, THP-1 was incubated with ADMA (0.1 - 10 μM) in a time-dependent manner and supernatants were harvested for the detection of proinflammatory cytokines by ELISA. The expression of MMP-9 by ADMA was determined by gelatin-zymography and northern hybridization.
Results: Tumor necrosis factor (TNF)-α was released at about 800 pg/ml within 12 h, whereas interleukin (IL)-6 was not. ADMA also stimulated the expression of monocytic chemotatic factors such as IL-8 and monocyte chemoattractant protein (MCP)-1. No effects were detected by symmetric dimethylarginine and *N*-monomethylarginine. ADMA induced the secretion of MMP-9 in a dose-dependent manner. To determine whether TNF-α mediated MMP-9 up-regulation, anti-TNF-α monoclonal antibodies were co-incubated with ADMA to block the action of TNF-α. Anti-TNF-α monoclonal antibody blocked completely the induction of MMP-9 by ADMA. **Conclusion:** This study suggests that ADMA induces the release of proinflammatory cytokines, TNF-α, IL-8 and MCP-1 in monocytes. The up-regulation of MMP-9 is mediated by TNF-α in ADMA treated monocytes. These findings provide the evidences that ADMA may be involved in the plaque formation and instabilization in atherosclerosis.

POSTER SESSION

1176 Nitric Oxide, Smoking, and Vascular Function

Tuesday, March 19, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: Noon-1:00 p.m.

Noon

1176-69 **Effects of Low Dose Hormone Replacement Therapy on Nitric Oxide Bioactivity and Markers of Inflammation Compared With Conventional Dose in Postmenopausal Women**

Kwang K. Koh, Mi-Seung Shin, *Gachon Medical School, Incheon, South Korea.*

Background: We have previously shown that conventional dose hormone replacement therapy (C-HRT) improved nitric oxide (NO) bioactivity and reduced markers of inflammation in postmenopausal women (PMW). The effects of low-dose hormone replacement therapy (L-HRT) has not yet observed.
Methods: We administered micronized progesterone (MP) 100 mg with conjugated equine estrogen (CEE) 0.625 (C-HRT) or 0.3 (L-HRT) mg daily for 2 months to 15 PMW with a washout period of 2 months in a randomized, double-blind, crossover study. Data= meanj¾SD. *=P<0.01, **=P<0.001 vs. Baseline.
Results: L-HRT and C-HRT significantly changed lipoprotein levels. L-HRT improved flow-mediated dilation (FMD) and tended to reduce plasma E-selectin , but not VCAM-1, CRP and fibrinogen levels compared with respective baseline levels. However, there were no significant differences between L-HRT and C-HRT regarding these effects. There were no significant correlations between absolute levels or percent changes of CRP levels and absolute levels or percent changes of FMD or E-selectin (all rjÂ0.155).
Conclusion: L-HRT has comparable effects to C-HRT in PMW regarding lipoproteins, FMD, and markers of inflammation.

	Baseline1	C-HRT	Baseline2	L-HRT
FMD (%)	4.80±1.26	7.14±1.75**	4.93±1.23	6.40±1.64**
E-selectin	41±9	37±10*	39±8	37±9
VCAM-1	468±172	484±179	464±175	442±168
CRP	0.26±0.21	0.30±0.31	0.19±0.15	0.19±0.09

1176-71 **Cyclosporine-Induced Hypertension and Vascular Superoxide Production**

Joseph N. Graziano, Carolyn L. Buller, John R. Charpie, *University of Michigan, Ann Arbor, Michigan.*

Background: Cyclosporine A (CSA)-induced endothelial dysfunction and systemic hypertension contribute to significant morbidity and mortality following orthotopic heart transplantation. Several experimental models of hypertension have recently been linked with endothelial dysfunction and increased vascular oxidant stress. We hypothesize that CSA increases vascular superoxide (O_2^-) production leading to a decrease in bioavailable nitric oxide, an endogenous vasodilator.
Methods: Adult male C57BL/6J mice (age 8-10 weeks) were treated for 12 days with either CSA (25 mg/kg in olive oil via intraperitoneal injection; n=12), vehicle (n=5), 1 mM Tempol (a superoxide dismutase mimetic administered in the drinking water; n=5) or CSA + Tempol (n=5). Systolic blood pressure (SBP) was measured daily by tail cuff method. Additionally, cultured human umbilical vein endothelial cells (HUVEC) were grown to confluence and incubated in phosphate buffered saline with 0.1 μM CSA (or ethanol vehicle) for ten minutes. Superoxide production was measured (n=4) by lucigenin-derived chemiluminescence (5 μM).
Results: CSA administration resulted in a significant increase in SBP from 113±5 mmHg (mean ± SEM) pretreatment to 184±2 mmHg on day 12 ($p<0.0001$). In contrast, co-administration of Tempol with CSA completely inhibited the CSA-induced increase in SBP (111±2 mmHg on day 12). Neither vehicle nor Tempol alone had any effect on SBP. Incubation of HUVEC with CSA resulted in a 15±1% increase in O_2^- production compared to HUVEC alone (p=0.02). Incubation of HUVEC with the endothelial nitric oxide synthase inhibitor N^{ω}-monomethyl L-arginine (L-NMMA, 1mM) resulted in an 11±2% increase in O_2^- production (p=0.04). Furthermore, there was a synergistic response to L-NMMA and CSA with an 85±10% increase in O_2^- production compared to HUVEC alone ($p<0.001$). Tempol (1mM) completely inhibited the increase in O_2^- in both CSA and CSA + L-NMMA treated cells.
Conclusions: CSA treatment increases blood pressure and endothelial cell O_2^- production. Inhibition of nitric oxide production augments the increase in CSA-induced O_2^- production, suggesting that excess O_2^- may scavenge nitric oxide and decrease its bioavailability.